

MORPHOLOGY AND CYTOGENESIS

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'In spite of the voluminous literature on the morphology of the pancreas, there is as yet no agreement on such a fundamental point as the recognition of cell types in the islets of Langerhans' (Thomas²⁷ 1937). During the past 21 years a considerable additional number of papers dealing with this subject have been published; the problem, however, remains largely unsolved.

Thus, according to Thomas, 'A, B and D cells have been identified by their differential coloration and constant nuclear qualities in the islets of all forty-one species of mammals examined' (including 10 species of rodents), whereas Jewell and Charipper¹⁷ could find no D-cells in the islets of the golden hamster. Gomori⁸ stated that 'there is no accepted routine procedure by which the cell types in the pancreatic islets can be identified with invariable certainty' and proposed a new modification of the Mallory-Heidenhain-azan stain suitable for the study of the islet cells. This was followed

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(Gomori⁸) by an improvement of the chromium-haematoxylin-phloxin method with which, according to Gomori, 'D-cells are indistinguishable from alphas.'

This lack of agreement on the recognition of cell types in the islets of Langerhans is overshadowed by the existing controversy on the *origin* of the cell types of the islets and the possible *transitions* between the different cell types found in the pancreas as a whole. The present investigation was undertaken in the hope that it would throw some light on these controversies.

MATERIAL AND METHODS

The animals used in this study were of the Wistar strain *Rattus norvegicus* bred in the Department of Physiology, University of Stellenbosch. For studying the different cell types in the islets of the albino rat, female animals weighing between 170-200 g. were used, except for 6 animals which were very young and weighed between 15-19 g. Some of these animals received injections of alloxan while others were

made diabetic by repeated injections of dextrose solution over a period of 21 days.

Regarding the study of the histogenesis and cytogenesis of the islets, embryos were collected at 6 different stages of development. Females in oestrus were placed with male animals at 10 a.m. and separated into individual cages at 2 p.m. The age of the embryos were arbitrarily computed from 12 noon.

Animals were killed by a sudden blow on the head and the pancreas or embryos quickly removed. The embryos were immediately decapitated and placed in the fixing solution. All materials were fixed in Bouin-solution for 10-12 hours, washed in running water for 8 hours and imbedded in paraffin wax. The adult material was sectioned serially at 3μ and stained according to Gomori's modification of the Mallory-Heidenhain-azan stain. The embryos were sectioned serially at 5μ and stained according to Gomori's improvement of the chromium-haematoxylin-phloxin method.

RESULTS

Experiment 1. An Investigation of the Different Cell Types found in the Islets of Langerhans of the Albino Rat.

In this experiment 30 rats were used. They were divided into 3 groups: Group A was the normal control group. The animals of group B were injected with a freshly prepared 3% solution of alloxan in distilled water. The animals of group

TABLE I. PROTOCOLS OF THE 30 RATS

Group	Rat No.	Body Weight (g.)	Injection and Dose	No. of Islet Sections Investigated	State of Islets	Cell Types
A	I	180	—	200	Normal	A & B
	II	176	—	200	"	A & B
	III	170	—	200	"	A & B
	IV	16	—	200	"	A & B
	V	17	—	200	"	A & B
	VI	170	—	18,206	"	A & B
	VII	175	—	19,107	Some degenerated	A, B & D
	VIII	180	—	17,983	Normal	A & B
B	IX	175	Alloxan-20 mg./kg.	200	Some degenerated	A, B & D
	X	180	"	200	"	A, B & D
	XI	190	body weight 3X daily	200	"	A, B & D
	XII	185	"	200	"	A, B & D
	XIII	175	for 21 days	21,288	"	A, B & D
	XIV	180	"	25,180	"	A, B & D
	XV	180	"	21,906	"	A, B & D
	XVI	170	"	22,679	"	A, B & D
	XVII	175	"	21,469	"	A, B & D
	XVIII	180	"	24,302	"	A, B & D
C	XIX	201	Dextrose-0.75 g./kg.	200	Some degenerated	A, B & D
	XX	187	"	200	"	A, B & D
	XXI	19	body weight 3X daily	200	"	A, B & D
	XXII	18	"	200	"	A, B & D
	XXIII	15	for 21 days	200	"	A, B & D
	XXIV	18	"	200	"	A, B & D
	XXV	180	"	23,741	"	A, B & D
	XXVI	180	"	22,730	"	A, B & D
	XXVII	175	"	22,139	"	A, B & D
	XXVIII	190	"	25,652	"	A, B & D
	XXIX	175	"	23,469	"	A, B & D
	XXX	180	"	22,804	"	A, B & D

C were injected with a 30% solution of chemically pure dextrose. The protocols of these animals are listed in Table I.

Two distinct cell types could be distinguished in the normal islets with the aid of Gomori's method (Fig. 1), except in rat No. VII (Table I) where a third type (which will be referred to as type D) was found. Although rat No. VII was included in this series as normal, some of the islets of Langerhans showed an advanced state of degeneration which was, however, only detected after the total number of islets in the pancreas was investigated (Fig. 2).

In the normal islet-section the central part is occupied by cells of irregular form with an average cross-section of 14μ .

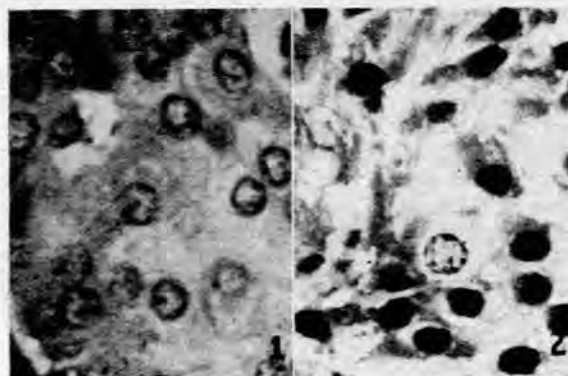


Fig. 1. Islet of Langerhans of normal rat. β -cells occupy the central part of the islet, while α -cells can be seen in the upper left-hand corner. X970. Fig. 2. Islet of rat No. VII (Table I). One 'D-cell' is shown among a number of degenerated β -cells with pyknotic nuclei. X970.

The cytoplasm stains a light orange-grey and is packed with fine dustlike cytoplasmic granules. These are considered to be β -cells. The second type is invariably found on the periphery of the islets, sometimes forming a complete ring encircling the β -cells, but often clumped together to form isolated groups on the periphery. They are on the average smaller than the β -cells, the cytoplasm stains somewhat more darkly and their cytoplasmic granules are more distinct with a faint red colour. These are considered to be α -cells. No difference in the structure of the nuclei of the α - and β -cells could be observed.

The 'D-cells' are characterized by being larger than both the α - and β -cells with an average cross-section of 18μ . The nuclei are also larger with apparently less chromatin-substance, which is usually arranged in irregular clumps against the nuclear membrane. In some of these cells the nuclear membrane itself is damaged and karyorrhexis has taken place. The cytoplasm appears to be very faintly stained and not filled with granules. Some cells of this type were found with large portions of the cytoplasm totally devoid of granules. However, where present, these granules could not be distinguished from those of the normal β -cells. They were therefore considered to be β -cells which had undergone hydropic degeneration.

In some islets of the animals in groups B and C, as well as in some of rat No. VII (group A), a considerable number of cells with pyknotic nuclei was found. These cells were smaller than the normal α - or β -cells and were considered to be β -cells which had undergone a marked degree of pyknotic degeneration, in contradistinction to the hydropic degeneration of the 'D-cells'.

From these observations it must be quite clear that the normal islets of Langerhans of the albino rat are constituted of two cell types only, while in those made diabetic, either by injection of alloxan or by a persistent elevation of the blood sugar by repeated intraperitoneal injections of dextrose, a third cell type ('D-cells') is invariably encountered apart from the fully degenerated β -cells showing pyknotic nuclei (Figs. 3 and 4).

Experiment 2. A Study of the Origin and Cytogenesis of the Islets of Langerhans

Normal healthy female animals were paired with males and killed at different times after successful copulation had

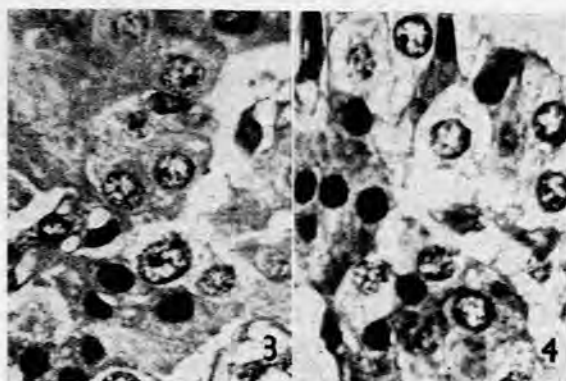


Fig. 3. Islet of alloxan-diabetic rat. Normal β -cells, 'D-cells' and degenerated β -cells with pyknotic nuclei can be distinguished. X970. Fig. 4. Islet of rat made diabetic by repeated injections of dextrose-solution; cells similar to Fig. 3. X970.

TABLE II. FINDINGS IN RAT EMBRYOS KILLED AT DIFFERENT TIMES

Rat No.	Killed after	No. of Islets	Total No. of Islet-cross-sections	Average Islet-Vol. c.mm. $\times 10^{-3}$	Total Islet Vol c.mm. $\times 10^{-3}$
XXXI	10½ days	None	—	—	—
XXXII	14½ days	—	—	—	—
XXXIII	17½ days	Islet-anlagen	—	—	—
XXXIV	19½ days	178	3,557	0.52	93.0
XXXV	21 days	233	4,787	0.57	132.3
XXXVI	22½ days	344	8,779	1.09	374.6

taken place (Table II). The embryos (one from each age-group) were serially sectioned at 5μ and all the sections, after having been stained with Gomori's modification of the chromium-haematoxylin-phloxin method, were microscopically examined with the aid of the oil-immersion objective. The total number of islets as well as the total number of cross-sections through these islets were counted. From this the average volume of the islets was calculated from the formula $4/3\pi r^3$. The diameter ($2 \cdot r$) was obtained by dividing the number of islet cross-sections by the number of islets (assuming that the islets are all spherical) and multiplying by 5μ i.e. the thickness of the sections. The following observations were made in this experiment:

At 10½ days. At this stage the ventral and dorsal pancreatic anlagen have developed and grown to such an extent that

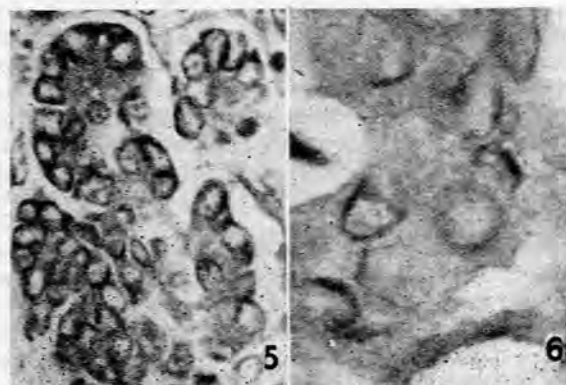


Fig. 5. Portion of pancreas of 17½-day embryo. An acinus-like structure is seen with centrally-situated cells similar to the centro-acinar cells of the adult pancreas. X450. Fig. 6. 'Primitive islet' from 17½-day embryo. It is seen in close relation to a pancreatic tubule. X970.

they have come in contact with each other, although complete fusion has not yet occurred. The cells are arranged in cords, some of which show distinct lumina (tubules) while others are nothing but solid masses of undifferentiated cells. Mitotic figures are very conspicuous, especially in the cells bordering the lumina of the tubules or those situated centrally in the solid cell cords. At this stage no structures are present that can properly be designated *islets of Langerhans*.

At 17½ days. The pancreas at this stage consists chiefly of tubules and solid cell cords embedded in embryonic connective tissue. Occasionally a tubule (now a secretory duct) ends in an acinus-like structure with one or more centrally-situated cells similar to the centro-acinar cells of the adult pancreas (Fig. 5). Mitotic figures are often present in these cells. Dispersed among these structures are found a few irregular masses of cells that are considered anlagen for future islets of Langerhans. Fig. 6 shows one of these *primitive islets* in close relation to a pancreatic tubule. Some of the cells can best be described as medium-sized with irregular shape and finely granular cytoplasm resembling that of the β -cells of adult islets of Langerhans.

Apart from these 'islets' a few cell masses are present that could easily be erroneously designated *primitive islets*. They are, however, nothing but conglomerates of connective tissue cells, as judged by their cytoplasmic processes. As seen from Table II the 'islets' were not counted because they were considered in no wise comparable to adult islets of Langerhans.

At 19½ days. Some of the 'islets' at this stage have developed to such a degree that they can be enumerated as distinct units. They should, however, not be considered 'islets of Langerhans' because they do not yet possess the two types characteristic of the mature islets—alpha cells are still absent.

At 21 days. More 'islets' are present, and the larger ones are more numerous (17% greater than 150μ in cross-section as compared to 12% at 19½ days). The average cross-section of the islets is, however, not appreciably greater than at 19½ days. This is due to the presence of a larger percentage of small, newly-formed 'islets'.

A very interesting phenomenon was noticed at this stage: several cells, showing beta granules, were found embedded among the cells of the pancreatic ducts, especially the intercalated ducts (Fig. 7). These are considered to be β -cells which differentiate directly from the duct-epithelium, in contrast to the β -cells previously encountered at the 17½ and 19½-days stage. These latter were invariably present among masses of undifferentiated cells (the primitive islets) and are considered to develop from these.

At 22½ days. Thirty-one per cent of the islets have now reached a size of 150μ or more in cross-section, and the total number of 'islets' has nearly doubled since the 19½-days stage. The total 'islet-volume' has more than doubled during this period. This enlargement of the islets must be attributed to mitotic activity within the islets—not only were mitoses observed in the undifferentiated cells of the primitive islets but mitotic figures are also found in the well-differentiated β -cells. The increase in the total 'islet-volume' is effected by enlargement of the existing islets as well as the development of new islets from the anlagen.

At this stage a *new cell type* also differentiates from the tubule-epithelium. Fig. 8 shows a cell of this type with characteristic alpha granules arranged in such a way as to

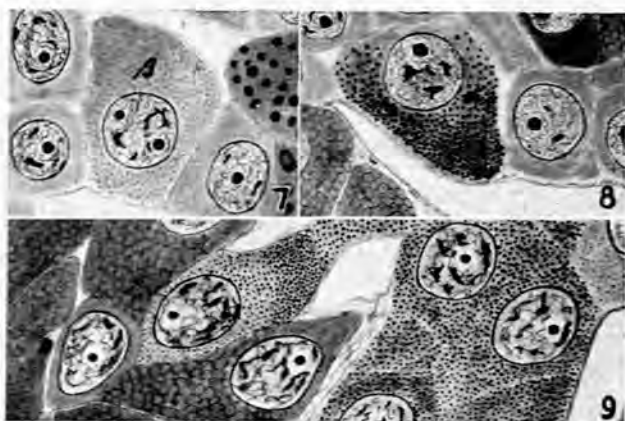


Fig. 7. Section through pancreatic tubule from 21-day embryo. A fully differentiated β -cell is shown. Fig. 8. Section through pancreatic tubule from 22½-day embryo. An α -cell with distinct polarity towards the capillary is shown. Fig. 9. Section of pancreas taken from rat treated with CoCl_2 . An acinus (left) adjacent to an islet (right) is shown. An α -cell, situated among the acinar cells and in contact with the centro-acinar cell, can be distinguished. (Figs. 7-9 were drawn at a uniform magnification; oil immersion.)

give it a distinct polarity towards the capillary and away from the lumen of the intercalated duct.

Addendum

During the course of an investigation, under way, on the destruction of the alpha cells by injection of CoCl_2 it was noticed that new α -cells are regenerated by differentiation from the centro-acinar cells. Fig. 9 shows an alpha cell situated between the cells of an acinus and still in contact with a centro-acinar cell, presumably the sister cell of the one under investigation. The polarity of this cell is similar to that of the α -cell described at the 22½-days stage.

DISCUSSION

The cell types in the islets of Langerhans. Laguesse²⁰ (1894) was the first to observe the existence of more than one cell type in the islets of Langerhans. Since then, different investigators paid attention to these different cell types and Lane²¹ succeeded in identifying two kinds of granules in the islet cells which differed chemically from each other and from the zymogen of the acini. These are now generally known as alpha and beta granules (present in the α -cells and β -cells, respectively). In addition to the α - and β -cells three other cell types were distinguished by different authors in different animal species: Bensley¹ described cells in the islets of the guinea pig and called them C-cells (gamma cells; Gomori⁹) and Bloom² (1931) described the so-called D-cells in the human islets of Langerhans. D-cells were found in 41 species of mammals (including the albino rat) by Thomas,²⁷ who also identified a fifth type of cell (E-cells) in the opossum islets.

With the staining methods of Gomori⁹ it was invariably possible to distinguish two cell types in the normal islets of the rat, except in some of the smaller islets in which only β -cells could be found. The differentiation of at least two cell types in adult pancreatic islets is further supported by their differential phosphatase activity (Gomori,¹⁰ Jacoby¹⁵ and McAlpine²²) and the electron-microscopic investigation by Lacy.¹⁹

Concerning the C-cells conflicting evidence appears to exist: Cowdry⁴ stated that 'A, B and C cells can be accepted without reservation as fundamental components of the islets'. According to Gomori,⁹ however, gamma cells (C-cells) are merely beta cells which are very poor in, or possibly devoid of specific granules. Bremer and Weatherford³ stated that 'the C-cell may represent a younger form of the A-cell'. E-cells were described in the islets of the opossum only. The D-cells, in my opinion, are nothing but cells in a certain phase of activity or degeneration, comparable to the β -cells undergoing hydropic degeneration. This opinion is in partial agreement with the statement of Gomori⁹ that 'D cells are probably aged alpha cells'.

The origin of the islets of Langerhans. The embryonic islets differentiate from the endoderm and do not show any genetic relationship to the mesodermal tissue in which they become embedded. The first 'islet-anlage' could be distinguished in the 17½-day embryo. No 'islets' were encountered in the 14½-day embryo, a finding which conflicted with Hard's statement¹² that the first 'islets' differentiated in the 13-day embryo. It must, however, be stressed that in the present investigation only those structures with a marked cellular compactness comparable to the adult islets of Langerhans, were counted as 'islets'.

From 17½ days onward 'islets' differentiated and increased in size at such a rate that at 22½ days the total volume reached the value of 374×10^{-3} c. mm. (Table II). This volume, however, constituted only a very small part of the islet-tissue of the adult as estimated by Haist and Pugh.¹¹ One might therefore conclude that only a small percentage of the future islets of Langerhans originate during intra-uterine development.

The cytogenesis of the β -cells. Gomori⁹ stated that 'the origin of beta cells is unknown'. Hard¹² was able to distinguish granules in the islet cells at the 19-day stage of embryonic development, while McAlpine²² described 'a new cell type' at 18 days and identified this type as the beta cell. In my material specific granules were first seen at 17½ days, which is in good agreement with the findings of McAlpine.

It was concluded that these first recognizable β -cells originated from undifferentiated cells which budded from the pancreatic ducts. Later on β -cells differentiated directly from the duct-epithelium, while some cells originated as a result of mitosis of existing β -cells. These results suggested a threefold origin of the β -cells. This conclusion conflicted, however, with that of Ferner and Stoeckenius⁶ who also claimed that three possibilities existed concerning the cytogenesis of the β -cells, but according to whom β -cells might originate *inter alia* by the transformation of α -cells into β -cells.

The cytogenesis of the α -cells. According to Hard¹² 'the beta cell is the only islet cell type to differentiate during embryonic development', and 'alpha cells are first recognized during the second day of postnatal life'. This opinion was not substantiated by the present study because occasional α -cells (with specific granules) were distinguished at 22½ days. Although Hultquist and Thorell¹⁴ believed that ' α -cells ... differ with respect to their ultraviolet cytology in budding germs and in islands of the adult type', I was unable to make this distinction.

In contrast to the suggested threefold origin of the β -cells, the α -cells showed a monophyletic origin *viz.* differentiation

from the duct-epithelium. Mitotic figures were never encountered in the α -cells of my material. This is supported by Jaffe,¹⁶ but conflicts with the finding of Mosca²³ who could see mitotic figures in both the α - and β -cells. Even after CoCl_2 -treatment the proliferation of α -cells in the adult occurred from duct-epithelium (the centro-acinar cells).

The relationship between the different cell types of the pancreas. A number of authors (Dale,⁵ Woerner,³⁰ and Johnson¹⁸) described transition-cells which showed characteristics of both islet- and acinar cells. From this they suggested that acinar cells could be transformed into islet-cells. Vincent,²⁹ Otani²⁴ and Sergeyeva²⁵ claimed that transformation of acinar cells into islet-cells and vice-versa occurred even in adults. On the other hand Tschassownikow,²⁸ working on the axolotl, stated that even the possibility of islets being transformed into glandular acini was excluded in the light of his observations. He therefore did not accept the 'theorie de balancement Laguesse'.

In the present study no transition-cells which showed characteristics of both acinar and islet-cells were found; it was concluded, therefore, that the islets of Langerhans were morphological entities *sui generis*. This conclusion is supported by Freise,⁷ Hirata¹³ and Gomori.⁹

Ferner and Stoeckenius' statement⁶ that α -cells can be transformed into β -cells is mistaken in my opinion. It is also in marked contrast to the findings of Terbrüggen,²⁶ Gomori⁹ and others. If the α -cells were to be taken as the precursors of the β -cells there is no possible explanation for the fact that the β -cells invariably differentiated during the embryonic development several days before there was any sign of specific α -granules (and thus of α -cells). Also, it will be very difficult to explain the observation that destruction of the β -cells with alloxan (Esterhuizen, 1948—unpublished results) is permanent, whereas destruction of the α -cells with CoCl_2 is followed by marked regeneration.

SUMMARY

1. The cellular composition of the islets of Langerhans in normal and diabetic rats was investigated. Rats were made diabetic by (a) injections of alloxan and (b) repeated injections

of dextrose solution. The normal islets of Langerhans of the albino rat are constituted of two cell types, while in those made diabetic a third type ('D-cells') was invariably encountered apart from the fully degenerated β -cells showing pyknotic nuclei.

2. Regarding the origin and cytogenesis of the islets it was found that the first 'islet-anlage', as well as specific beta-granules, could be distinguished in the 17½-day embryo. Alpha-cells were first distinguished at 22½ days. It is suggested that the β -cells have a threefold and the α -cells a monophyletic origin.

3. No transition-cells which showed characteristics of both acinar and islet-cells were found. It is concluded, therefore, that the islets of Langerhans are morphological entities *sui generis*.

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All photomicrographs were taken through a Wratten 'K' filter.

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